FINAL REPORT

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PRINCIPAL INVESTIGATORS: Drs. Mahendra K. Jain and J. Gregory Zeikus

INSTITUTION: Michigan Biotechnology Institute, Lansing, MI.

GRANT TITLE: Extreme Biocatalyst Culture Collection for Unique Microorganisms

AWARD PERIOD: 27 August 1994 - 31 December 1997

OBJECTIVE: To develop an Extreme Biocatalyst Culture Collection (EBCC) as a resource center to supply pure, viable and authentic cultures of extremophilic microorganisms which are non-conventional, novel, or of extreme nature; to improve various long-term preservation methods for these types of cultures.

APPROACH: The EBCC will maintain a specialized collection of diverse cultures which grow under extreme environmental conditions and which include species that are obligately thermophilic, acidophilic, alkalophilic, halophilic, osmophilic and/or anaerobic including methanogens. These will also include dehalogenating cultures and those that utilize toxic gases such as carbon monoxide as substrate. Long-term preservation methods such as liquid nitrogen, lyophilization, etc. along with revival techniques will be examined and developed for these cultures. The EBCC will supply, on payment, these cultures to the scientific community for their research work. EBCC will work closely with ATCC.

ACCOMPLISHMENTS : The EBCC has been developed into a small culture collection that has collection of diverse cultures from extreme environments. A data base has been developed using Microsoft Access to systematically organize the EBCC cultures. This data base meets the current needs of the EBCC. Inventory of all the existing cultures has been completed into the new data base. A brochure on the collection for distribution to the public has been developed. This brochure provides information about the EBCC along with contact name, address and the telephone/fax numbers. The brochure was distributed at professional meetings such as annual meetings of the American Society for Microbiology, and Society for Industrial Microbiology in 1996 and 1997, and at the 8th International Congress for Culture Collection, Veldhoven, The Netherlands, 1997.

A meeting of the EBCC Advisory Committee was held in the early period of the grant (July 26, 1995) to get input from the experts. The committee members included Robert Gherna (ATCC, Rockville, MD), Richard Starr (Algal Culture Collection, Texas), John Breznak (Michigan State University, East Lansing, MI), Juergen Wiegel (University

of Georgia, Athens, GA), Greg Zeikus and Mahendra Jain (EBCC, MBI, Lansing, MI). Among the several recommendations, the committee advised that EBCC should first complete cataloging of all the existing cultures into a new data base in order to adequately handle the culture acquisition and supply requests. It was also suggested that initially the cultures could be accepted even if these were not fully characterized. During the grant period, contacts with individual scientists were made for gradual acquisition of the cultures. Additional halophilic and psychrophilic cultures from marine environments were acquired. At the same time, pure and authentic cultures were supplied to scientists at universities and other organizations on demand. All the cultures received for acquisition were processed and checked before entering into the EBCC catalog and putting them aside for a long-term storage.

Three commonly used preservation methods were evaluated for long-term maintenance of extremophilic cultures. Three cultures representing each of the psychrophilic, halophilic and thermophilic group of organisms were randomly selected. These were *Bacillus globiosporus* (EBCC 1707), *Bacillus psychrophilus* (EBCC 1708), *Micrococcus cryophilus* (EBCC 1706) representing psychrophiles, *Halobacteroides halobius* (EBCC 1703), *Haloanaerobium prevalens* (EBCC 1315), *Haloincola saccharolytica* (EBCC 1642) representing halophiles, and *Thermococcus litoralis* (EBCC 1690), *Thermotoga maritima* (EBCC 1692), *and Thermoanaerobacterium aotearoense* (EBCC 1818), representing thermophiles. The cultures were healthy and in log phase when preserved at -70°C, in the liquid nitrogen, and in the lyophilized form. The results are only available for a 24-month storage period. Based on the colony forming units, cryopreservation in liquid nitrogen and freezing at -70°C were found to be better than lyophilization for all the selected cultures in this study except for *Thermococcus litoralis,* cells of which were more viable in a lyophilized form than when preserved by freezing at -70°C in glycerol. Evaluation of these methods will be continued for long-term storage of these cultures for five and ten year periods.

A temperature revival study was conducted on the three psycrophiles; *Micrococcus cryophilus, Bacillus globisporus, and Bacillus psychrophilus.* Vials stored in liquid nitrogen were revived at two temperatures (25°C, 37°C). Total viable counts were done and plates counted after 6 days incubation at 10°C. Two out of the three organisms showed higher viable counts when thawed at 25°C. *Micrococcus cryophilus* showed no difference in viability number for the two temperatures. This study shows that the traditional method of fast thaw at 37°C may not be the best method for organisms of extreme nature.

The database developed last year has been continuously updated with information on the cultures currently maintained in the EBCC collection. The information includes but is not limited to culture source, number of vials, location of vials, media, growth requirements, and references. An EBCC catalog of cultures is ready and will be periodically updated when collection starts growing.

CONCLUSIONS: All the existing cultures have been catalogued into the new database system. Additional cultures were acquired and distributed. The common preservation methods can be adapted to preserve the microbial cultures from extreme environments. Only long-term preservation results will indicate the survivability of cultures under different preservation conditions. A catalog of the existing cultures has been prepared.

SIGNIFICANCE: There is a tremendous increase in efforts on isolation of novel microorganisms which have extreme physicochemical growth requirements. These extremophilic microbial cultures have been shown to be very important in both environmental and industrial processes. As a result, the demand for these types of cultures has increased. However, since these extremophilic microorganisms require very different and novel techniques for their growth and maintenance, organization of a resource center for such cultures remain a challenge.

PUBLICATIONS, PRESENTATIONS AND ABSTRACTS:

1. Extreme Biocatalyst Culture Collection - A brochure published in 1995.

2. Jain, M.K. Growth and maintenance of halophilic anaerobes. Presented at the Workshop on Halophilic Anaerobes, October 31 - November 1, 1995, University of Goa, Goa, India.

3. Jain, M.K., D. Burgdorf and J.Z. Zeikus. Extreme Biocatalyst Culture Collection - a source for unique microorganisms. Presented at the 8th International Congress for Culture Collection, Veldhoven, The Netherlands, 1997.

4. Burgdorf, D. and M.K. Jain. 1998. Evaluation of preservation methods for selected cultures from extreme environments. (Manuscript under preparation for submission).

5. Jain, M.K., D. Burgdorf, and J.G. Zeikus. 1998. Catalog of Cultures, Extreme Biocatalyst Culture Collection (complete with media and index).